Insecticidal Properties and Chemical Composition of *Conyza Aegyptiaca* (L.) Oil: Studies on Two Dipterous Insect Pests

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Abstract: The herb, *Conyza aegyptiaca* L., was subjected to hydrodistillation process to extract the essential oil from the whole dry plant material. Larvae and adult stages of the mosquito, *Anopheles pharoensis*, and the housefly, *Musca domestica*, were used as test model organisms representing two dipterous insect pests of medical importance. Under standard bioassay test methods, the LC50 of the oil accounted to 37.8 ppm and 0.087 mg/cm^2 against larvae and adults of *An. pharoensis*, respectively. These toxicity parameters were found to be 71.8 ppm as LC50 and 0.125 µg/insect as LD50 against larvae and adults of *M. domestica*, respectively. Using GC/MS analysis, we identified 19 compounds constituting ca. 97% of phytochemicals present in the oil, such as monoterpenes, sesquiterpenes and esters. Limonene constituted about 50% of the plant oil (48.79%), followed by (E) - β-Ocimene (8.66%), Germacrene D (7.54%) and β-pinene (6.91%). The occurrence of the other constituents ranged between 0.27% and 5.29%. It was concluded that the potency of *C. aegyptiaca* oil refers mainly to the presence of limonene. The findings of this study may encourage more research aiming at investigation of eco-friendly biopesticides based on botanical resources.

Keywords: *Conyza aegyptiaca,* Essential oil, Limonene, Mosquitoes, Housefly.

INTRODUCTION

The order Diptera presents an array of insects which being more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases. One such insects, which share a close ecological niche with man is the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae). Apart from disease transmission, *M. domestica* soils man's food and usually constitutes a nuisance, particularly the adult stage [1]. House flies, occur throughout the tropics and are also found in warm temperate regions and some cooler areas. It is recognized as a serious public health pest to human beings and livestock by transmitting many infectious diseases causing a serious threat to human and livestock by vectoring several pathogenic organisms such as protozoa cysts, helminth parasites, enteropathogenic bacteria, and enterovirus [2-5].

Also, mosquitoes are responsible for the biological transmission of several diseases like filariasis, dengue fever, Japanese encephalitis and malaria. Despite an array of control measures taken to suppress mosquito populations, they continue to flourish and contribute to high human mortalities, particularly in developing countries [6].

Indeed, the use of insecticides remains the first line of defense against herbivorous insects, nematodes, plant pathogens and insect vectors of disease. Control measure against these insects in the short-term still depends on the use of conventional insecticides [7, 8]. The indiscriminate use of chemical insecticides has given rise to many well-known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment [9]. Insecticide resistance in house fly and mosquitoes is a global problem and several surveys have shown that such resistance is wide spread and increasing [10, 11]. These problems coupled with the high cost of chemical pesticides have stimulated the search for biologically based alternatives. Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. They tend to have broad-spectrum activity, relative specificity in their mode of action, and easy to process and use. They also tend to be safe for animals and the environment [12]. Specifically, many studies have drawn attention to the toxic effects of plant extracts on related Diptera [8, 13, 14].

Our concern here is directed to one candidate plant of the Asteraceae family, *Conyza aegyptiaca* L. The genus *Conyza* (Asteraceae) is an annual, biennial or perennial herbaceous plant. It consists of 80 – 100 species growing in tropical and subtropical areas of the world [15, 16]. *C. aegyptiaca* is an aromatic herb mainly distributed in Africa, tropical Asia and Australia [16]. The plant is used in folk medicine as an anthelmintic, a body-wash for convalescents, and a soothing for skin diseases [17]. Previous pharmacological studies have shown that its polar extracts possess antiviral and antimicrobial activities [18].

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Recent studies were conducted in our laboratory [19-21] to screen the insecticidal activities against the mosquito, *Anopheles pharoensis* and the housefly, *Musca domestica* for ethanolic crude extracts of some plant species including *C. aegyptiaca.* To the best of our knowledge, however, except our previous studies, the literature offers no data on the insecticidal activity of *C. aegyptiaca* against the above mentioned insects. Also, there are no data on the efficacy of its essential oil against mosquitoes and housefly. Therefore, in continuation to our goals, the current work was undertaken to estimate toxicity of the essential oil against larval and adult stages of the mosquito, *Anopheles pharoensis* and the housefly, *Musca domestica*. Also, to identify the oil constituents that may responsible for its insecticidal activity.

MATERIALS AND METHODS

Plant

The herb (*C. aegyptiaca* L.) was collected from road side at 6th October City, Giza, Egypt and authenticated by Prof. M. El- Gebaly, Flora Dept., National Research Centre, Cairo, Egypt. The fresh herb was washed, air dried, powdered and kept in dark glass bottle until used for extraction.

Test Insects

Anopheles pharoensis: An. pharoensis were reared under standard conditions at 27ºC, 70% relative humidity and a 12L: 12D photoperiod. Eggs were placed in plastic trays filled with distilled water. Larvae were reared at a fixed density of 100 larvae per tray and fed upon tetramine, which is recommended for larval development and female fecundity [22]. Pupae were collected from day 11 to 15, placed in emergence cages of 50 × 50 x 50 cm and provided with a piece of sponge supplied with 10% glucose solution that was suspended by a wire thread from the roof of the cage. Adult females were fed on pigeons. The eggs laid were transferred to small plastic containers filled with distilled water and a small amount of tetramine. After breeding for several generations, 4th instar larvae as well as 5-day-old female adults were selected for running bioassay tests.

Musca domestica:Musca domestica (MD) house flies were reared in the insect rearing room of our laboratory at 25-27°C, and 55-60% relative humidity. A standard rearing method [23] was adapted to provide 3rd larval instars and adult flies of 0-24hrs old for running bioassay tests.

Extraction of Plant Oil

Volatile oil was obtained by hydrodistillation using Clevenger apparatus [24]. Hundred grams of dried whole plant material of *C. aegyptiaca* were powdered and placed in a 2 L flask filled till its half with tap water and distilled over an electric sand path. At the end of distillation (usually 3-5hrs.) the sand path was shut off and the resulted oil was withdrawn from the apparatus tap in a small vial, dried over anhydrous sodium sulfate for removing any water droplets, filtered, weighed and kept in a deep freezer (-18°C) till used in bioassay tests and phytochemical analysis.

Toxicity Bioassay

I. Tests with mosquitoes

a. **Larvicidal Activity.** Standard methods for testing the susceptibility of mosquito larvae to insecticides [25] were followed with minor modifications. A series of 7 concentrations of the tested oil were prepared in 250ml glass beakers, marked at 100ml level, each containing ca. 90ml of distilled water. The desired amounts of oil were added to the beakers followed with few drops of Tween-80 to facilitate solubility of oil. Ten newly moulted 4th instar larvae of *Anopheles pharoensis* were transferred to each beaker in least quantity of water by means of a small dropper, and then the solution level was adjusted to the 100ml mark. This process was repeated in three other beakers for each tested concentration. In control experiments, plain water containing few drops of Tween-80 was used. All beakers were incubated at room temperature for 24 h and then mortality was recorded. Percentage mortalities were corrected according to Abbott's formula [26]. The probit analysis was used to estimate toxicity values (e.g., LC50 & LC95 values), as well as the slope of regression lines according to Finney [27]; using LD-P Software program.

b. **Adulticidal Activity:** The test was conducted by dissolving a definite amount of the plant oil (0.0 up to 12.0 mg) in 2 ml acetone and spread on the surface of a strip of filter paper of 15 × 5 cm size equivalent to a concentration of (0.0 up to 0.16 mg/cm^2). After drying, the strip was rolled inside a glass vial of 8 × 5 cm size. Ten females of *An. pharoensis* (5-days-old) were anaesthetized by CO_2 and transferred to the vial by aspirator, and then the vial was covered. After recovery (about 20 min.), the females were allowed to be in contact with the strip for 1 h. Control tests were carried out in the same manner but the filter paper was impregnated with 2 ml acetone free of plant oil (i.e., 0.0 concentration). After the specified duration (1 h) the adult females were transferred into wooden cages (20 × 20 × 20 cm), containing cotton pieces soaked with water, and the percent kill was recorded after 24 h. The mortality was adjusted by Abbott's formula [26], and the toxicity values were estimated according to Finney [27]; using LD-P Software program. Usually, a series of 7 concentrations were prepared to estimate the toxicity values. The procedure used was that of Wright [25].

II. Tests with Housefly

a. **Larvicidal Activity.** Standard methods for the evaluation and testing of new insecticides [25] were followed with minor modifications. Larvicidal tests were based on exposing *M. domestica* (MD) larvae to food-contaminated with toxicants (i.e., "bait"). The bait was prepared by mixing 2g coarse wheat bran with 2ml water containing the tested oil. Third larval instars of MD were allowed to feed on batches of freshly prepared baits placed in 250 ml - glass bakers; each provided with 10 larvae. Eight concentrations of 4 replicates each were usually tested for the plant oil along with control treatment containing bait free of plant oil. All beakers were

incubated at room temperature for 24h, then percent mortalities were estimated and corrected according to Abbott's formula [26]. Probit analysis [27] was performed to estimate toxicity values (e.g., LC50 & LC95) and slope of regression lines; using LD-P Software program.

b. **Adulticidal Activity.** Standard methods for the evaluation and testing of new insecticides by topical application [25] were followed with minor modifications. The houseflies were anaesthetized with diethyl ether for 5 minutes where 1µl of the test solution was applied by a Clinical Series pipette (CSP) on the dorsal thorax of 0-24 h-old adult housefly of mixed sexes selected randomly. Ten insects were used for each concentration. Eight concentrations of 4 replications each were usually tested along with control treatments dosed with solution free of the tested oil. All beakers were incubated at room temperature for 24 h, then percent mortalities were estimated and corrected according to Abbott's formula [26]. Probit analysis [27] was performed to estimate toxicity values (e.g., LD50 & LD95) and slope of regression lines; using LD-P Software program.

Analysis of the Plant Oil

The obtained oil from *C. aegyptiaca* was subjected to Gas Chromatography/Mass Spectrometry analysis (GC/MS) coupled to a HP5973 mass-spectrometer to identify its chemical constituents. Samples of 0.5µl of the essential oil diluted in n-hexane were injected manually in the splittless mode into Hewlett Packard Chromatograph Model 5970, equipped with flame ionization detector (FID) and 20 meter HP capillary column (0.2 mm I.D.). The oven temperature was programmed at 3ºC/min. from 60 ºC to 200 ºC, and then isothermally at 200 ºC for 25 min. Detector and injector temperatures were 250ºC and 200ºC, respectively. Helium was used as carrier gas at a constant flow of 1.0 mL min^{-1}. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 40-750 m/z and a scan interval of 0.5 s. The identification of the components was based on comparison of their mass spectra with those of NIST3.0 Libraries provided with the computer-controlling GC-MS system as well as from the published literature.

RESULTS

Mortalities resulted from exposing the larvae and adults of *Anopheles pharoensis* to different concentrations of *Conyza aegyptiaca* oil is presented in Table **1**. Mortalities in either larval or adult stages of the tested mosquito occurred in a concentration- dependent manner. Exposure of mosquito larvae to a range of concentrations (20-70 ppm) induced mortalities ranged between 20-90%. In mosquito adults, the oil concentration of 0.07 mg/cm^2 caused ca. 21% mortality which was increased gradually up to 91.7% at a concentration equivalent to 0.12 mg/cm^2 (Table **1**).

In housefly tests, mortalities in either larval or adult stages of the tested *M. domestica* insect were also occurred in a concentration- dependent manner. Complete mortality (100%) in housefly larvae occurred from exposing to bait containing oil at a concentration of 110 ppm (Table **2**). Topical application for the housefly adults by 0.16 µg plant oil to each insect achieved 95.7% mortality.

The obtained concentration- mortality data (Table **1**) enabled us to estimate the toxicity values of *C. aegyptiaca* oil against both insects as shown in (Table **3**). It appeared that the oil possessed LC50 and LC95 values accounting to 37.8 and 108.7 ppm, respectively against the mosquito larvae; values extrapolated from a regression line of 3.6 slope. Such toxicity values in case of the mosquito adults were 0.087 and 0.133 mg/cm^2, respectively for LC50 and LC95 derived from a regression line of 9.1 slope value.

By the same manner the obtained concentration- mortality data (Table **2**) revealed the following toxicity values for

Table 1. Mortalities Resulted from Exposing of 4th Instar Larvae and Adult Females of *Anopheles pharoensis* to Different Concentrations of *Conyza aegyptiaca* Oil

Concn. (ppm)	%Mortality Against Larvae	Concn. (mg/cm^2)	%Mortality Against Adults
Cont.	0.0	Cont.	0.0
20	20.0	0.07	20.9
30	35.9	0.08	33.7
40	47.1	0.09	56.0
50	60.0	0.10	68.7
60	77.3	0.11	80.0
70	90.0	0.12	91.7

Table 2. Mortalities Resulted from Exposing 4th Instar Larvae and Adult Stage of *Musca domestica* to Different Concentrations of *Conyza aegyptiaca* Oil

Concn. (ppm)	%Mortality Against Larvae	Concn. (µg/insect)	%Mortality Against Adults
Cont.	0.0	Cont.	0.0
50	20.0	0.10	15.1
60	33.7	0.11	27.3
70	45.0	0.12	39.9
80	57.9	0.13	50.3
90	70.0	0.14	70.0
100	83.1	0.15	81.3
110	100.0	0.16	95.7

Table 3. Toxicity Values for Conyza Aegyptiaca Oil Against Larvae and Adult Stages of *An. pharoensis*[a] and *M. domestica*[b]

An. Pharoensis Larvae			*An. Pharoensis Adults*		
LC50 (ppm)	**LC95 (ppm)**	**Slope[c]**	**LC50 (mg/cm^2)**	**LC95 (mg/cm^2)**	**Slope[c]**
37.8	108.7	3.6	0.087	0.133	9.1
M. Domestica Larvae			*M. Domestica Adults*		
LC50 (ppm)	LC95 (ppm)	Slope[c]	LD50 (µg/insect)	LD95 (µg/insect)	Slope[c]
71.8	139.3	5.7	0.125	0.172	11.9

[a] Data refer to Table **1**.
[b] Data refer to Table **2**.
[c] Slope of regression line.

the tested oil against the housefly (Table **3**):

I. Larval toxicity values: LC50 = 71.8 ppm; LC95 = 139.3 ppm; slope value = 5.7.

II. Adult toxicity values: LD50 = 0.125 µg/insect; LD95 = 0.172 µg/insect; slope value = 11.9.

In the present study, we identified 19 compounds constituting ca. 97% of phytochemicals present in the oil, such as monoterpenes, sesquiterpenes and esters. It was found that limonene constitutes about 50% of the plant oil (48.79%), followed by (E) - β-Ocimene (8.66%), Germacrene D (7.54%) and β-pinene (6.91%). The occurrence of the other constituents ranged between 0.27% and 5.29% (Table **4**).

DISCUSSION

Conyza aegyptiaca is one of the most used plants to treat malaria in Rwandan traditional medicine. It is also used in the treatment of haematuria [28]. The author reported that methanolic leaf extract of the plant showed a moderate antiplasmodial activity (IC_{50} = 22.7 µg/ml) and was slightly more active than the dichloromethane leaf extract (IC_{50} = 36.8 µg/ml). The methanolic leaf extract showed a low cytotoxicity (IC_{50} = 80.9 µg/ml) but presented a low selectivity index (3.6). To the best of our knowledge, however, the literature offers no data on the insecticidal activity of *C. aegyptiaca* oil, a matter which was targeted in the present study in an attempt to fill a gap in this direction.

Essential oils and their volatile constituents are widely used in the prevention and treatment of human illnesses. They are also documented for exhibition of acute toxicity, anti-feeding and oviposition deterrents against a wide variety of insect-pests [29, 30]. Certain plant species containing essential oils have proved efficacy as larvicides, adulticides, ovicides and repellents against different species of mosquitoes [31-36]. The lower risk level of the volatile essential oils to the environment and their minimal residual activity against predator, parasitoid and pollinator insect populations, making essential-oil-based pesticides compatible with integrated pest management programs [30, 37].

Table 4. GC/MS Analysis of *Conyza aegyptiaca* Oil

No.	Chemical Name	Retention Time (min)	Peak Area (%)	Chemical Formula	Molecular Weight
1	α-pinene	7.18	0.90	$C_{10}H_{16}$	136
2	Sabinene	7.32	0.72	$C_{10}H_{16}$	136
3	β-pinene	8.04	6.91	$C_{10}H_{16}$	136
4	Myrcene	8.72	1.64	$C_{10}H_{16}$	136
5	Limonene	9.02	48.79	$C_{10}H_{16}$	136
6	(Z)- β-Ocimene	9.82	0.27	$C_{10}H_{16}$	136
7	(E)- β-Ocimene	10.03	8.66	$C_{10}H_{16}$	136
8	(E)-Caryophyllene	10.41	4.36	$C_{15}H_{24}$	204
9	α-Humulene	12.53	0.49	$C_{15}H_{24}$	204
10	β-Farnesene	12.81	0.83	$C_{15}H_{24}$	204
11	Germacrene D	15.94	7.54	$C_{15}H_{24}$	204
12	Bicyclogermacrene	16.21	5.29	$C_{15}H_{24}$	204
13	δ-Candinene	16.40	3.54	$C_{15}H_{24}$	204
14	Trans-Nerolidol	17.45	1.02	$C_{15}H_{26}O$	222
15	Germacrene D4-ol	17.53	0.91	$C_{15}H_{26}O$	222
16	Spathulenol	18.31	1.64	$C_{15}H_{24}O$	220
17	Caryophyllene oxide	19.35	2.06	$C_{15}H_{24}O$	220
18	Epi-α-Muurolol	20.15	0.61	$C_{15}H_{26}O$	222
19	α- Cadinol	21.48	0.81	$C_{15}H_{26}O$	222
Total identified peak area%			96.99%		

Phytochemical investigations on *C. aegyptiaca* have led to the isolation of diterpenes [38]; triterpenes [39, 40]; sesquiterpenes [38, 41, 42]; flavonoids [43, 44]; and Phloroglucinol Glucoside derivative, roseoside and kaempferol-3-*O*-*β*-D-glucopyranoside [45] from the aerial parts of the plant**.** In the present study, we identified some phytochemicals present in the oil**.** As mentioned above, the triterpene limonene constituted about 50% of the plant oil (48.79%) (Table **4**). This may give an indication that *C. aegyptiaca* is a limonene-rich plant, compared with some other plants. For instance, essential oil of dill seeds was reported to be 9.34% [46]; and (44.1% or 16.6% or 16.0%) in *Anethum graveolens* seeds; according to results published by other investigators [47-49]. But citrus limonene exceeded these levels, and was reported to range between 51.97% in Sour Orange oil and 95.32% in Lime oil [36].

The insecticidal activity of plant oils was extensively referred to limonene content in the essential oils [36, 50-52]. Both Kassir *et al*. [53] and Mohsen *et al*. [54] reported the larvicidal action of limonene against the larvae of Cx. quinquefasciatus. According to Mansour *et al*. [36], the Lime peel oil of 95.32% limonene content showed the highest toxicity against *Culex pipiens* larvae (LC_{50} = 77.6 ppm), while Sour Orange peel oil of 51.97% limonene content exhibited the lowest toxicity (LC_{50} = 136.4 ppm). This dose-dependent trend was not fully obvious with the results obtained from the adulticidal tests. For example, Mandarin peel oil of 73.11% limonene content showed LC50 of 0.105 mg/cm^2, compared to 0.155 mg/cm^2 for Grapefruit peel oil which contained 93.08% limonene. In a comparison held between toxicity of limonene alone with the citrus peel oils, it was concluded that limonene alone has a basic biocidal properties against the tested insect, but its potency can be improved considerably when being in the oil extracted from citrus peels; due to possible synergistic interaction with the other compounds in the oil **(**Mansour *et al*., 2004**).**

From the above mentioned, we suggest that the obtained toxicity results of *C. aegyptiaca*-essential oil may refer to limonene which was dominated above all the other detected compounds. Limonene, as a pure compound was reported to possess LC50 of 126.1ppm and LC50 of 0.165mg/cm^2, respectively against larvae and adult stages of *Culex pipiens* mosquitoes [36]. According to the results of the present investigation, the essential oil from *C. aegyptiaca* showed superior toxicity against the larvae (LC50 = 71.8ppm; Table **3**) and the adults (LC50 = 0.087 mg/cm^2; Table **3**) of *An.*

pharoensis. Such results may be attributed to different susceptibility of both mosquito species, as well as different compositions of the tested substances in both cases compared and possible synergistic action exhibited by the oil rather than the pure compound limonene; based on Mansour *et al*. [36].

The results in Table **3** indicate that the slope values equaled 3.6 and 5.7 in larvicidal tests with *An. pharoensis* and *M. domestica*, respectively. Such values equaled 9.1 and 11.9, respectively in the adulticidal tests. Simply, these slope values indicate that the larval and adult stages of *M. domestica* are more vulnerable than the larval and adult stages of *An. pharoensis* to the toxic action of *C. aegyptiaca* oil. According to Busvine [55], with a steep regression line (i.e., of high slope value), the test insect shows obvious response to each little increase of the toxic dose, and the opposite with a regression line of low slope value. Also, as high slope of a regression line as more homogeneity of the tested insect population to the tested toxicant.

In conclusion, the results of the present study provide new data focusing on the insecticidal efficacy of the essential oil extracted from the native herb, *Conyza aegyptiaca*, against pests of medical importance such as mosquitoes and housefly. The potency may refer mainly to the presence of limonene in the oil. Such findings may encourage more research aiming at investigation of eco-friendly biopesticides based on botanical resources.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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None Declared.

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